A SIMPLE PROCEDURE FOR PREPARATION OF COLLOIDAL GOLD FOR DIAGNOSTIC PURPOSES.

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The first attempts to prepare colloidal gold in St. Luke's laboratory were made according to the procedure recommended by Miller, Brush, Hammers, and Felton,[1] but in spite of the greatest care the solutions obtained were uniformly poor. Eventually it was decided to experiment independently with a view of getting some insight into the trouble which we and many of our predecessors had experienced, and ultimately a reliable and exceedingly simple technic was evolved for preparing colloidal gold which will satisfy every clinical requirement.

The glassware used was the Pyrex brand. It was cleaned, with no more attention than that usually given to laboratory apparatus, and was rinsed only with tap water. Indeed, it was noticeable that solutions with which it came in contact did not wet the surface but tended to form in drops. It is advisable, however, to avoid beakers that have had bad scratches, as reduction tends to take place there.

Ordinary distilled water was used; no attention was paid to the time that elapsed since distillation, and it was drawn from stock containers. In view of the impurities possibly contained in some of the reagents[2] and the known toleration of colloidal gold solutions for electrolytes,[3] it is felt that in an emergency tap water could be employed, and satisfactory solutions have been made with it. All the reagents were obtained from Merck & Co. The potassium carbonate and oxalic acid were of the "Blue Label Reagent" quality,

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[1] A Further Study of the Diagnostic Value of the Colloidal Gold Reaction, together with a Method for the Preparation of the Reagent, Bull. Johns Hopkins Hosp., 1915, xxvi, 298.

[2] The formaldehyde always contains formic acid. Ten c.c. of the formalin used required 1.1 c.c. of $\frac{N}{10}$ NaOH for neutralization (phenolphthalein). The maximum limits of foreign substances in Merck's "Blue Label Reagent Potassium Carbonate" used were stated to be:

Heavy metals	0.0000	per cent.
Chlorides (Cl)	0.0020	"
Sulphates (So$_3$)	0.0075	"
Nitrates (N_2O_5)	0.0800	"
Potassium cyanide (CN)	0.0480	"
Sulphides (S)	0.0200	"
Sulphites (SO_3)	0.0080	"
Thiosulphates ($K_2S_2O_3$)	0.0800	"
Phosphates (P_2O_5)	0.0040	"
Silicates	0.0000	"
Aluminum (Al)	0.0400	"
Calcium (Ca)	0.0010	"

[3] 1.7 c.c. of 1 per cent. NaCl is necessary to precipitate completely 5 c.c. of colloidal gold in one hour. Five c.c. of colloidal gold will remain unaffected by 1 c.c. of 0.4 per cent. NaCl for an indefinite period.

the formalin U. S. P.; but there would seem to be no objection to using any good grade of chemicals convenient. The gold chloride was in sealed ampoules, and it is noteworthy that the weight of "gold chlorides" contained therein varies very considerably.[4]

The solutions were of the following strengths:

Gold chloride acid	1 per cent.
Potassium carbonate	2 "
Oxalic acid	1 "
Formaldehyde (37 per cent. CH_2O)	2.5 per cent (5 c.c. formalin made up with 200 c.c.).

The solutions should preferably be freshly made, although all but the last keep very well. The oxalic acid probably does not deteriorate at all, but the potassium carbonate slowly tends to dissolve its container and incorporate silicates; the gold chloride is easily reduced upon exposure to dust or light, and the formaldehyde undergoes marked changes from oxidation, polymerization, etc.

It was assumed that since good solutions had been prepared with 10 c.c. of 1 per cent. $AuCl_3$ per liter, that that amount might not be considered excessive, provided that reduction was not carried too far. Keeping this constant, it was found that an increase of potassium carbonate tended to give violet and purple solutions. This was the experience of Miller, Brush, Hammers and Felton, and so the original amount of 7 c.c. was retained. These solutions, however, were invariably cloudy and more or less opaque. (Ten drops (0.43 to 0.58 c.c.) of oxalic acid and not more than 5 c.c. of formaldehyde were used.) The amount of oxalic acid was then gradually increased and a marked improvement was at once apparent. The solutions were very much more transparent and the minimum amount necessary to obtain the maximum transparency seemed to be about 1.75 c.c. This was also in accordance with the results obtained at Johns Hopkins, but the statement that "a large amount of oxalic acid without the subsequent addition of formaldehyde made clear 'satt rot' colloidal gold solutions" could not be verified. The solutions obtained in this laboratory were beautifully clear, but always blue or purple, although the conditions were varied within wide limits.

Finally, the formaldehyde factor was studied. It was evident from the behavior of the oxalic acid not only that the formaldehyde was essential to secure the ruby red color, but that it was responsible for the brown turbidity.[5] Beginning, then, with 5 c.c. the amount was slowly reduced (1.75 c.c. of oxalic acid being used), and although for a long time no change could be noted in the appearance of the solutions, this very fact was proof that too much formaldehyde was being employed. Very suddenly, though, almost ideal solutions resulted, which, moreover, responded perfectly to known paretic spinal fluids. Flesch notes that he could not obtain good solutions unless he used much less than 10 c.c. of 1 per cent. formalin, usually 2 or 3 c.c.[6] The proper amount seems to be about 0.83 c.c. of 2.5 per cent., formalin containing 37 per cent., by weight of CH_2O, or 8.07 mg. of CH_2O per liter of colloidal gold made up.

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[4] The contents of two tubes labeled as containing 15 grs (972 mgs.) of "Gold Chlorid Merck Acid" contained respectively 824 and 869 mgs.

[5] It is suggested that the brownish turbidity is caused by a suspension of reduced gold of almost molecular dimensions. Solutions which are fogged invariably deposit a brown precipitate transmitting violet light and resembling purple of Cassius and the violet gold precipitated from colloidal gold solutions by electrolytes. Such particles may have been caused by reduction by trioxymethylene (paraformaldehyde), and we have observed that when the Ostwald pipette used in measuring the formaldehyde is allowed to remain on the desk several hours, an amorphous white precipitate can be seen in the tip, and if the pipette is used a very cloudy solution results, whereas a clean dry pipette gives clear solutions.

In order, then, that the correct amount of formaldehyde should be used it is necessary to determine the value of the formalin. This may be conveniently done by a modification of the iodometric method of Ronijn,[7] which we have worked out as follows:

EVALUATION OF FORMALIN. Ten cubic centimeters of the formalin is made up to 1 liter with distilled water and 5 c.c. of this solution is mixed with 25 c.c. of decinormal iodin. Two normal sodium hydroxide is added drop by drop until the liquid becomes clear yellow. After ten minutes 2 c.c. of concentrated hydrochloric acid are added and the free iodin titrated with decinormal sodium thiosulphate. Two atoms of iodin are equivalent to one molecule of formaldehyde. If T equals the titration and S the specific gravity of the formalin, then

$$\text{Percentage of } CH_2O = \frac{75 - 3T}{S}$$

Up to this point the gold and alkali had been added at 60°, the oxalic acid at 80°, and the formaldehyde at 90°. The experiment was made of introducing all the chemicals initially, keeping the temperature just high enough to cause gradual reduction. The results were eminently satisfactory, and we have since been guided entirely by the color changes rather than by any definite temperature. This preliminary mixing of all the units of the reacting system insures their perfect and uniform distribution and eliminates the unfortunate and unavoidable local reduction effect entailed by the seriatim method of introduction of the reducing agents.

The following procedure has been finally adopted, and although it is not *guaranteed to be infallible in giving ideal solutions*; with a little experience it will be found not at all difficult to get 90 per cent. of the trials to answer every clinical requirement. As the cost of the chemicals is less than five cents per liter, and a dozen solutions may be prepared in an afternoon, it is no extravagance to discard even 50 per cent. of these should this be necessary.

TECHNIC OF SECURING ONE LITER OF "SATT ROT" COLLOIDAL GOLD. To 1 liter of distilled water in a clean flask add 10 c.c.

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[6] Flesch: Ztschr. f. d. ges. Neurol. u. Psychiat., 1914, xxvi, 318.

[7] Ztschr. Anal. Chem., 1897, xxxvi, 18.

of 1 per cent. gold chloride, 7 c.c. of 2 per cent. potassium carbonate, 1.75 c.c. of 1 per cent. oxalic acid, and 0.83 c.c. of 1 to 40 formalin (37 per cent. CH_2O) or equivalent amount of 8.07 mg. of CH_2O per liter. Mix thoroughly, cover with a watch-glass, turn the burner on full and heat with occasional stirring to between 80° and 85°. Then reduce the flame enough to keep the temperature constant. The wire gauze supporting the beaker should be covered with a sheet of asbestos cardboard to prevent local reduction on that portion of the beaker directly over the flame. The solution will slowly develop through a series of colors substantially as given below, though some of the shades are much more transient than others. The associated temperatures are merely typical and were recorded for a development which lasted about an hour.[8]

When the solution reaches its maximum depth of color a remarkable lightening and change of color occurs within the space of a few seconds, and *until this rebound and transition of color has taken place the solution will be worthless for clinical purposes.* After this point has been passed, the burner may be turned out and the solution allowed to cool to room temperature, although if wanted at once there is no objection to cooling in running water.

If a solution is heavily clouded, pour it out and examine the flask for spots and streaks of reduced gold transmitting green, blue or violet light. If noticeably present, add a little aqua regia,[9] cover with a watch-glass and warm for a few minutes. Then rinse thoroughly and repeat the experiment. A clear colloidal gold solution leaves a clean container.

If the solutions still continue to be turbid and show a strong brownish fluorescence a series of 6 liters should be made with amounts of formaldehyde, decreasing and increasing by 0.05 c.c. from that originally used, and notice taken in which direction

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[8] Kaplan observed that the color change comes gradually as a rule in good colloidal gold solutions: Ztschr. f. d. ges. Neurol. u. Psychiat., 1915, xxvii, 246.

Color.	Temperature.
Faint blue green	65°
Pale blue green	74°
Pale bluish lavender	78°
Bluish lavender	79°
Pinkish lavender	80°
Bluish pink	82°
Pale pink	83°
Pink	84°
Deep pink	84°
Violet red	84°
Bluish red	83°
Deep bluish red	83°
Deep ruby red	83°
Dark ruby red (violet red in test-tube or pipette)	83°
Rebound or color transition	83°
Orange red (orange pink in test-tube or pipette)	83°

[9] Seven volumes of concentrated hydrochloric acid in two volumes of concentrated nitric acid.

improvement occurs. We have observed that too little formaldehyde will cause cloudiness as well as too much.

It is perhaps advisable to keep the solution in the dark, but it is not necessary to keep it cold, and so far as we know the solution once made will keep indefinitely, certainly for several months. Contrary to the generally accepted requirement a slight brownish surface-dispersion was found to make no difference in the practical application of the colloidal gold. The practice of Kafka[10] of rejecting all solutions which are tinged blue may be adopted, and it may also be *stated for the guidance of those making colloidal gold that the correct* color is duplicated almost exactly (in a test-tube comparison) by a solution of 5 c.c. of 20 per cent. by weight of cobaltous nitrate ($Co(NO_3)_2.6H_2O$) plus 0.3 c.c. of 0.25 per cent., potassium bichromate ($K_2Cr_2O_7$) solution, plus 10 c.c. of water.[11]

A certain amount of black sediment often accumulates on the bottom of the colloidal gold solution, but this does not impair its usefulness.

The procedure given above applies to the preparation of 1 liter of colloidal gold at a time, and it is not advisable to experiment with quantities greater or less than that amount until some experience has been gained.

None of the solutions prepared by this method were of the protected type, and all of them gave typical curves not only with general paresis but with lues, tabes, and meningitis. No attention was paid to the question as to whether the final solution was neutral or not, but 5 c.c. of every solution prepared was completely precipitated in one hour by 1.7 c.c. of 1 per cent. sodium chloride, and no change of color whatever could be detected with normal spinal fluids.

In an article by H. S. Hulbert[12] the inherent disadvantages of formalin are discussed and a neat method is shown of avoiding these by utilizing formaldehyde vapor, oxalic acid being omitted, and the course of the reaction being guided by the color of the solution.

The tubes used for the clinical tests were heavy glass, lipless test-tubes, measuring $\frac{11}{16}$ in. to $\frac{3}{4}$ in. x 6 in. They are not readily broken in washing, and the point of the 1 c.c. pipette may safely be pressed against the bottom of the tube in order to avoid air bubbles and danger of spinal fluid being carried into the mouth of the operator. After using they are rinsed in tap water and immersed overnight in a 1 to 10 dilution of aqua regia. This effectually dissolves any precipitated gold which may be sticking to the glass, and which it is difficult otherwise to remove.

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[10] Kafka: Deutsch. med. Wchnschr., 1913, xxxix, 1874.

[11] It is the intention to publish later the composition of a complete permanent standard colloidal gold color scale blended from inorganic constituents similar to the above, and based upon the colors observed with pathological spinal fluids.

[12] Technical improvement in Lange's colloidal gold test, Jour. Michigan State Med. Soc., January, 1916.

The 1 c.c. pipettes should be graduated to the tip, otherwise two measuring points must be determined and time lost by returning the amount in excess of 1 c.c. to the preceding tube. It is well to see, too, that there is a wide margin between the 1 c.c. mark and the top of the pipette.

Although in the usual technic the concentration of the spinal fluid in the tenth tube is 1 to 5120, the ratio of the total volume to the volume of the colloidal gold employed is higher than in the other tubes, unless 1 c.c. is removed from the tenth tube and rejected. This point is not clearly brought out in the descriptions of the technic which have come under our notice, with the result that the curves obtained before making this correction had an upward "kick" at their ends which was rather misleading.

CONCLUSIONS. 1. That colloidal gold solutions which conform to every clinical requirement (differential paretic, luetic, tabetic, meningitic, and normal curves) may be made with comparative ease.

2. That it is quite unnecessary to use other than ordinary distilled water from any convenient laboratory still.

3. That nothing is to be gained by elaborate precautions in cleaning the glassware used, and that apparatus washed with soap and water, rinsed in tap water, and hung up to dry has been found entirely satisfactory.

4. That the reagents used may all be introduced at the beginning of the experiment, and temperatures other than the final one disregarded.

5. That providing sufficient oxalic acid is present the only critical points involved are the amount of formaldehyde used,[13] and the passing of the color transition point at which the solution lightens and becomes orange red.

6. That solutions so prepared were of the proper color and transparency and precipitated properly with a dilute electrolyte.

7. That if a thick layer of the solution is transparent, and of the proper color, a certain amount of surface dispersion is no bar to its usefulness.

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THE PRESENT STATUS OF HODGKIN'S DISEASE.[1]

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The past five years have witnessed a wave of renewed interest in the subject of Hodgkin's disease. Inasmuch as the crest of this wave seems to have been reached, and possibly passed, it is perhaps

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[13] This is in the vicinity of 17 per cent. of that recommended by Miller, Brush, Hammers and Felton.

[1] Read before the Chicago Society of Internal Medicine.